a reporter gene, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

REMARKS

I. Status of the Claims

All claims previously pending are still currently pending. Claims 234–241 and 243 have been amended to recite that the promoter is selected from the group consisting of a cellular promoter and eukaryotic viral promoter. Support for this amendment can be found on page 7 of the present specification. Claim 58 has been amended to replace the phrase "amplifiable marker" with the phrase "sequences encoding an amplifiable marker" to point out that the amplifiable marker refers to DNA sequences on the vector construct. Claim 59 has also been amended to recite that the vector contains a sequence "encoding" an amplifiable marker. Claim 59 has also been amended to recite that the vector does not comprise a homologous targeting sequence. Support for this amendment can be found throughout the specification, for example, on pages 30–31 of the present specification. Claim 86 has been amended to particularly point out that the vector is introduced into a cell *in vitro* prior to being expressed *in vivo*. Support for this amendment can be found throughout the present specification, for example, on page 8, first paragraph. Claim 98 has been similarly amended. Claim 157 has been amended to recite that the selectable marker sequence "encodes" a selectable marker. Other amendments address rejections on the grounds of insufficient antecedent basis. Accordingly, no new matter has been added.

II. Miscellaneous

On page 3 of the Office Action, claims 90–97, 99–104, 107, 108, 128 and 131 have been objected to as being improper multiple dependent claims. Claim 90 depends on claim 89, which depends from claim 87 or claim 232. The same applies to claims 91–97, 99–104, 107, 108, 128 and 131. Applicants have amended claim 89 so that it is not dependent upon claim 232. Accordingly, these claims can be treated on the merits.

On page 3 of the Office Action, the Examiner has objected to claim 59 for failing to limit the subject matter of a previous claim. Claim 259 should depend upon claim 257. This claim has, accordingly, been amended.

Accordingly, it is believed that the Examiner's objections have been addressed and overcome. Reconsideration and withdrawal of the objections and treatment of claims on the merits is, therefore, requested.

III. The Rejections

A. The Rejection Under 35 U.S.C. § 101

On page 3 of the Office Action, claims 234–259 have been provisionally rejected under 35 U.S.C. § 101 for claiming "the same invention" as claims 58–60 and 64–69 of co-pending U.S. Application No. 09/515,123. Applicants respectfully traverse the rejection.

The Examiner indicates that claims 58–60 and 64–69 of the co-pending application are the same as claims 244, 246, 251, 253 and 255–257 of the present application. Accordingly, the Examiner acknowledges that not all of the rejected claims (234–259) are properly rejected. Furthermore, only some of the claims specifically designated above are the same claims as in the present application. Specifically, claims 246 and 259 are not identical. Claim 259 recites that the construct does not contain a targeting sequence. Accordingly, these claims are not co-extensive in scope. With respect to claims that may be the same (58, 68 and claims dependent thereon), upon resolution of the other outstanding issues in this case, claims will be deleted in one of the two cases.

B. The Rejection Under 35 U.S.C. § 112 First Paragraph: Written Description

On page 4 of the Office Action, claims 234–259 have been rejected under 35 U.S.C. § 112, first paragraph, as lacking adequate written description. Applicants respectfully traverse the rejection.

In the present case, Applicants submit that the specification does reasonably convey possession of the invention in claims 234–259. First, Applicants direct the Examiner to Applicants' Supplemental Amendment and Remarks filed July 14, 2000, specifically to pages 9-11. Here, the Applicants cited proof of adequate support. The Examiner has not discussed his reasons for concluding that Applicants' support is inadequate. If the rejection is maintained, Applicants request that the Examiner discuss, with specificity, his reasons for concluding that

Applicants' evidence for support is inadequate. Nevertheless, Applicants now discuss grounds for adequate support in further detail.

Prior to discussion, Applicants point out that for purposes of response to this rejection, *Applicants cite their earliest priority application, 09/941,223*, filed September 26, 1997. For the Examiner's convenience, a copy of this specification is attached as Exhibit A. Applicants cite their earliest priority application and provide a copy for the Examiner because the Examiner's finding of a lack of support has been used to reject the claims under 35 U.S.C. § 102(b) by Zambrowicz *et al. (Nature*, 392:608–611 (1998)). Support in Applicants' 1997 specification is the relevant support to address the rejection. The Examiner has also rejected the claims under 35 U.S.C. § 102(e) over a U.S. patent filed on April 8, 1998, based on a provisional application filed on March 27, 1998. Accordingly, Applicants show support in their 1997 priority application to address this rejection.

As the Examiner states on page 4 of the Office Action, the test for sufficiency of support is whether the disclosure "reasonably conveys to the artisan that the inventor had possession at that time of the later-claimed subject matter."

Applicants note that to legally comply with the written description requirement, it is not necessary that the application describe the claimed invention in the exact words of the claim. What is required is that the application reasonably conveys to persons in the art that, as of the filing date, the inventors had possession of the subject matter claimed. For example, a

species in a genus or specific permutations need not be expressly disclosed. It is sufficient that the person of ordinary skill in the art would have recognized that the inventors intended the invention to encompass the species or permutation. Further, support can be shown by drawings, as well as by text.

On page 4 of the Office Action, the following limitations are alleged, without reasons, to lack sufficient support in the specification: (1) vector does not contain a polyA site operably linked to the transcriptional regulatory sequence; (2) exon does not encode antibiotic resistance; (3) exon is not a reporter gene; (4) no selectable marker between the transcriptional regulatory sequence and the splice donor sequence; (5) exon does not contain a selectable marker; (6) no ribosomal entry site between the transcriptional regulatory sequence and the splice donor sequence; (7) exon does not contain an internal ribosome entry site; (8) exon is derived from a naturally-occurring eukaryotic gene; and (9) splice donor sequence is derived from a naturally-occurring eukaryotic splice donor sequence. Applicants address each of these in turn.

Prior to discussing support for each of the elements, Applicants submit that the specification conveyed various combinations of the elements. For example, splice donor, IRES, exon, and selectable marker are all optional. Therefore, the person of ordinary skill in the art would have readily envisaged vectors containing or lacking one or more of these elements.

Furthermore, the specification would have reasonably conveyed to the person of ordinary skill in the art that alternative embodiments of the elements are encompassed in the invention. These alternative embodiments, for the specific elements at issue, are presented in detail below.

PolyA Signal

The Examiner states that claim 234 lacks support for a vector that does not contain a polyA site operably linked to the transcriptional regulatory sequence. Applicants submit that the specification would have reasonably conveyed this aspect.

Figure 1 shows the transcriptional regulatory sequence operably linked to a eukaryotic exon bordered by an unpaired splice donor sequence. In the diagram, the splice donor allows splicing onto exon II in an **endogenous** EPO gene. The polyadenylation sequence is shown on the **endogenous** exon V. This figure alone reasonably conveys the absence of a polyadenylation signal operably linked to the transcriptional regulatory sequence; a polyadenylation signal downstream of the vector-encoded splice donor site would interfere with the diagrammed events.

Further, selectable activation vectors are discussed in the paragraph spanning pages 17 and 18, and on page 18. The text indicates that the polyA site will be supplied by the endogenous gene for the vector construct shown in Figure 4.

Exon Does Not Encode Antibiotic Resistance

The specification provides optional embodiments wherein the exon encodes a selectable marker. The existence of an alternative embodiment would reasonably convey the other alternative embodiment. For example, on page 20, construct number 18 is designated as a regulatory sequence linked to a selectable marker followed by an unpaired splice donor site. Page 25 also discusses the use of selectable markers in the vector exon sequence as one alternative. See the paragraph spanning pages 25 and 26. The paragraph spanning pages 26 and 27 also set forth the option of a selectable marker in the exon. In addition, on page 28, first full paragraph, it is indicated that a screenable marker can be used in place of a selectable marker. In addition, the third full paragraph of page 28 indicates that a selectable marker may be omitted from the construct. Still further, both Figures 1 and 4 show that the Applicants clearly disclosed embodiments wherein the exon does not contain a selectable marker. Applicants submit that by providing an **option** wherein the exon encodes a selectable marker, it would be immediately apparent that this is an option and that, therefore, vectors are also intended in which the exon does not encode a selectable marker. Selectable markers are specifically, though not exhaustively, listed in the paragraph spanning pages 27 and 28. Antibiotic resistance genes, for example, neomycin resistance and puromycin resistance, are exemplified. The most common form of selectable marker used in the art was antibiotic resistance. Thus, antibiotic resistance would also have been reasonably conveyed to the person of ordinary skill in the art.

Exon is Not a Reporter

Applicants direct the Examiner to the constructs shown on pages 19–21, particularly to construct number 20. These constructs show that one of the options for the exon is that it contains a reporter gene. The fluorescent green protein is an example of a reporter gene encoded in an exon. Further, the paragraph spanning pages 25 and 26 indicates that the exon can encode an epitope tag or screenable marker. Screenable markers for the exon are also in the paragraph spanning pages 26 and 27. Screenable markers would have been recognized as reporters in that they allow detection without drug or other selective pressure. Examples of screenable markers are found in the first full paragraph of page 28, and include cell surface proteins, fluorescent proteins, and enzymes. Still further, both Figures 1 and 4 show that the Applicants clearly disclosed embodiments wherein the exon does not contain a reporter gene. Since a reporter gene is an option, this reasonably conveys a vector in which the exon is other than a reporter.

No Selectable Marker Between the Transcriptional Regulatory Sequence and Splice Donor Sequence

The specification is directed to the option wherein the exonic sequence between the transcriptional regulatory sequence and splice donor sequence can be a selectable marker. By providing this embodiment as an option, the specification reasonably conveys the embodiment of not having a selectable marker as the exonic sequence between the transcriptional regulatory sequence and splice donor sequence. See the paragraph spanning pages 25 and 26, wherein the regulatory sequence is operably linked to an exon, which in one

embodiment could be a selectable marker, and wherein a splice donor may optionally be present immediately 3′ of the exon sequence. The paragraph spanning pages 26 and 27 further discusses the possibility that the exonic sequence is a selectable marker sequence. Further, the second full paragraph of page 28 refers to the activation construct lacking a selectable marker. The following paragraph also refers to a construct as lacking the selectable marker.

Still further, both Figures 1 and 4 show that the Applicants clearly disclosed embodiments wherein the vector did not contain a selectable marker between the transcriptional regulatory sequence and the splice donor sequence.

No Ribosomal Entry Site (IRES) Between the Transcriptional Regulatory Sequence and the Splice Donor Sequence

The specification clearly discloses embodiments where the exon contains an internal ribosome entry site (IRES) and where it does not. Because of these alternative embodiments, Applicants submit that the specification reasonably conveyed to the person of ordinary skill in the art that the Applicants had possession of an embodiment of the vector wherein the exon does not contain an IRES.

An IRES is well-known and used generally to initiate translation of mRNA internally. Thus, it would have been appreciated by the person of ordinary skill in the art that initiation of translation could occur, by means of an IRES, on a downstream cistron. Accordingly, the person of ordinary skill in the art would have understood that Applicants'

vectors can encompass an IRES when a downstream sequence on a chimeric transcript is translated. This occurs, for example, where the vector exon contains a selectable marker or other gene containing a stop codon. A chimeric transcript is produced between the exon gene sequence and the endogenous sequence. Translation is terminated with the stop codon. Accordingly, in order to obtain translation of the endogenous sequence, an embodiment disclosed throughout Applicants' specification, internal initiation of translation of the endogenous gene can be achieved by the use an internal IRES. In other embodiments, however, no IRES is necessary because the vector exonic sequence does not contain an open reading frame flanked by a start codon and a stop codon. Thus, including an IRES is only an alternative embodiment. As to placement of the IRES, the specification teaches that it would be located on the vector 3′ to the transcriptional regulatory sequence, but 5′ to the splice donor sequence if it is used.

Claim 6 refers to the vector constructs of any of claims 2–5 containing an IRES for producing a polycistronic message. This means that the vectors of claims 2–5 need not contain an IRES.

On page 18, first full paragraph, the embodiment is included wherein an internal ribosome entry site is included between the transcriptional regulatory sequence and the unpaired splice donor site. This text refers to Figure 4 as having this embodiment. It is indicated that with this embodiment, the internal ribosome entry site allows protein translation to initiate at a coding exon of the endogenous gene that lacks a start codon. A further example is shown in the

constructs listed on pages 19–21, construct numbers 18 and 19. Accordingly, the person of ordinary skill in the art would have recognized that the vectors of invention may or may not include an IRES.

Exon is Derived From a Naturally-Occurring Eukaryotic Exon

In the paragraph spanning pages 25 and 26, the second sentence indicates that the exon may be a naturally-occurring sequence or may be non-naturally-occurring. Further, it is indicated that the codons in the vector exon may be the same as the codons present in the first exon of an endogenous gene to be activated. This information clearly indicates that the exon sequence can be derived from a natural eukaryotic exon sequence. In addition, the paragraph spanning pages 25–26, clearly indicates that the codons can be derived from a naturally-occurring gene.

The specification contains various instances of the use of eukaryotic exons. Figure 1 also shows instances of the use of a eukaryotic exon between the transcriptional regulatory sequence and the splice donor sequence, specifically, the human growth hormone exon.

Accordingly, Applicants respectfully submit that the specification reasonably conveys to the person of ordinary skill in the art that the Applicants had possession of the embodiment wherein the exon is derived from a naturally-occurring eukaryotic exon.

Splice Donor Sequence is Derived From a Naturally-Occurring Eukaryotic Splice Donor Sequence

Applicants submit that the specification would have reasonably conveyed a naturally-occurring splice donor sequence. Page 48 of the Applicants' present specification discusses a splice donor sequence. In the second full paragraph, the specification has provided a consensus splice donor sequence which would be, by definition, eukaryotic, since splicing does not occur in prokaryotic cells. This consensus sequence provides for naturally-occurring splice donor sequences. Examples of naturally-occurring eukaryotic splice donor sites matching the consensus site were known in the art. For example, AAg gTA AgT, (one permutation of the consensus splice donor site disclosed in the specification) is found in the factor VIII exon IV splice donor sequence (published 1994). CAG GTG AGT occurs in the EPO exon IV (published 1993). Thus, Applicants submit that the specification would have reasonably conveyed to the person of ordinary skill in the art that the Applicants were in possession of the embodiment wherein the splice donor sequence is derived from a natural eukaryotic splice donor sequence.

The function of the written description requirement is to ensure that the inventor had possession of the claimed subject matter. It is not necessary that the application describe the limitations *ipsa verbis*. What is necessary is that persons of ordinary skill in the art will reasonably recognize that Applicants had possession of the limitations at issue. Furthermore, the U.S. Patent and Trademark Office has the burden of showing that the limitations are not adequately described. Thus, it is up to the U.S. Patent and Trademark Office to provide reasons why the description is insufficient. Accordingly, if this rejection is maintained in the face of the

text that the Applicants have presented and their discussion regarding that text, Applicants respectfully request that the Examiner provide reasons why this disclosure does not provide adequate support.

In view of the text discussed above, Applicants respectfully submit that the specification of the earliest filed priority document reasonably conveyed the claimed limitations to persons skilled in the art.

C. The Rejection Under 35 U.S.C. § 112 First Paragraph: Enablement

On page 5 of the Office Action, claims 58, 59, 64–69, 71, 76–80, 85–89, 98, 105, 108, 109–123, 129, 157, 180–183, 223–226, and 232–259 have been rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the claims are not enabled for sequence elements linked in any other order than that specifically recited. Applicants respectfully traverse the rejection.

On page 6 of the Office Action, the Examiner discusses the case in which the amplifiable marker is downstream from the splice donor site. He indicates that the marker would be spliced out when the splice donor is spliced with a splice acceptor site of an endogenous gene. This is not accurate. Splicing reactions, like any reaction, are not 100% efficient. Occasionally, splicing does not occur. As shown in the art, this can be exploited to allow amplification. U.S. Patent No. 5,561,053 (attached as Exhibit B) teaches the placement of an amplifiable marker 3′ of a splice donor site and 5′ of a splice acceptor site. Within the cell, a certain percentage of transcripts fail to splice out the amplifiable marker. The low level of amplifiable marker

expression allows the artisan to select for amplification of the vector. Thus, it was known in the art that an amplifiable marker could be positioned 3′ of a splice donor site and still be functional.

The Examiner specifically discusses only claims 58, 59 and 157. To the extent that the rejection applies to claims 232–259, however, Applicants point to U.S. Patent No. 6,080,576, cited by the Examiner in the Office Action (attached as Exhibit C). Applicants direct the Examiner to claim 5. This claim is not limited to a specific order for the splice donor and marker components and has been considered enabled by the U.S. Patent and Trademark Office. The Applicants' present claims should also, therefore, be enabled on this basis alone. However, where, as in claim 244, a second promoter precedes the marker sequence, transcription will proceed independently from the first and second promoters. Accordingly, even if there is splicing in a transcript initiated at the first promoter, independent transcription will occur from the second promoter operably linked to the marker. Thus, translation from this independent transcript will occur regardless of whether splicing occurs from an upstream splice donor sequence.

With respect to the vector in claim 59, there is no splice donor recited. Applicants submit that amplification can be achieved irrespective of the position of the amplifiable marker on the construct. With respect to the viral origin of replication, the same applies. The function of a viral origin of replication is such that wherever it is located on the vector, it can achieve its intended function without interfering with the other functions of the recited components on the vector.

In claim 157, the orientation of the components is already recited. There is already a limitation that the positive and negative selectable markers and splice donor are oriented in the construct such that when the vector is integrated into the genome and an endogenous gene is transcriptionally activated, the positive selectable marker is expressed in active form and the negative selectable marker is either not expressed or is expressed in inactive form. This means that the positive selectable marker could not be spliced out by the splice donor. If it was, then it could not be expressed in active form when the endogenous gene is transcriptionally activated. Accordingly, contrary to the Examiner's assertion ("if one put the splice donor first, all the marker genes would be spliced out"), the limitations would prevent this embodiment from occurring. On the other hand, the claim would clearly cover the embodiment in which the negative selectable marker is in fact spliced out by the splice donor site. This is one embodiment in which, upon transcriptional activation of the endogenous gene, the negative selectable marker is not expressed.

On page 6 of the Office Action, the Examiner objects to a functional embodiment wherein an enhancer is linked to a splice donor "or other sequence elements". The Examiner does not explain "other sequence elements." Accordingly, Applicants cannot duly respond. Clarification is requested as to "other sequence elements."

On page 7, the Examiner specifically discusses the case in which the enhancer is operably linked to a splice donor sequence or amplifiable marker or both. He maintains that the

endogenous gene would not be expressed because "such an enhancer would have not initiated transcription of the gene." Applicants point out that the claims encompass embodiments wherein a transcriptional regulatory sequence, including an enhancer, is operably linked to an unpaired splice donor sequence. This means that the enhancer enhances expression levels of a transcript containing the unpaired splice donor sequence.

The enhancer would not *initiate* transcription. With respect to the amplifiable marker, the presence of an enhancer can enhance expression of an endogenous gene, and this enhancement does not interfere with the function of the amplifiable marker either 5′ or 3′ to the enhancer. Conversely, the amplifiable marker would not interfere with transcriptional enhancement from the enhancer.

The Examiner has merely posed these questions, but has not indicated why an amplifiable marker should interfere with enhancement of an endogenous gene or that enhancement of an endogenous gene should interfere with amplification by means of the amplifiable marker. Applicants submit that such interference would not occur. If the Examiner disagrees, Applicants request that he provide specific, scientific explanation regarding why believes that this interference should occur.

A further ground for rejection is that the claims are enabled only for eukaryotic cells. The claims have been amended to recite host eukaryotic cells and methods using these cells.

On page 7 of the Office Action, the Examiner rejects the claims on the grounds that the methods cannot be practiced *in vivo*. The Examiner asserts that activation of a gene or over-expression of a gene *in vivo* by providing cells containing an activation vector is highly unpredictable "because of the multitude of factors." The Examiner states that, at the time of the invention, "both *in vivo* gene therapy methods, as well as *ex vivo* gene therapy methods were unpredictable." Several references are cited to support this position.

Applicants point out that the claims are not drawn specifically to gene therapy but generally to expression of the activated gene *in vivo*. Moreover, the disclosure generally discloses *in vivo* expression by the activated cell. Although this encompasses *ex vivo* cell therapy, the disclosure is not so limited. Expression would also encompass well-known utilities, such as production of useful proteins in an animal, for example for manufacturing, or transgenic animals useful for phenotypic screening or drug screening *in vivo*. Even with a non-transgenic animal, cells implanted into an animal by adoptive transfer could also be used for drug screening or for phenotypic screening in the live animal. Attached hereto as Exhibit D are references showing *in vivo* gene expression of implanted genetically-modified cells.

Asakuno et al. (Reference 1) reports the transplantation of normal rat kidney fibroblasts transfected with a human genomic metallothionein promoter/human genomic c-fos fusion gene, producing c-Fos protein, into the striatum of rat brain. The transplanted cells were

still viable after eight weeks. The transplanted cells continued expressing the c-fos transgene, resulting in the promotion of cell growth and neovascularization.

Nagashima et al. (Reference 2) describes the transduction of natural killer cells with the interlukin-2 gene and the implantation of such cells into tumor-bearing mice. The reference shows that the cells could be used to provide anti-tumor activity against established three-day liver metastases. Stable expression of the transgene in the natural killer cells was thus showed in tumor-bearing host. The transduced natural killer cells secreted sufficient quantities of bioactive interlukin-2 to mediate anti-tumor effects *in vivo*.

Mathisen et al. (Reference 3) describes lymph node cells transfected with a DNA construct containing interlukin-10 under the control of an inducible interlukin-2 promoter. Upon adoptive transfer, transfected T-cell clones inhibited the onset of an experimental autoimmune disease.

Chen et al. (Reference 4) reports the implantation of primary fibroblasts modified to secrete nerve growth factor into the brain of memory-impaired rats. The fibroblasts were shown to survive for six weeks and to continue expressing nerve growth factor mRNA. This expression provided significant amelioration of memory impairment and increased the size and number of nerve growth factor receptor-positive neurons. Further, implantation of these cells into normal young adult rats resulted in significant memory impairment and hypertrophy of low affinity nerve growth factor receptor-positive neurons.

Garver et al. (Reference 5) reports the transplantation of mouse fibroblast secreting recombinant human alpha 1-antitrypsin into peritoneal cavities of nude mice. Expression was shown after a four week evaluation in different tissues.

Petersen et al. (Reference 6) describes the production of human transferrin in recombinant fibroblasts that were implanted into athymic mice. Transduced cells were implanted subcutaneously into athymic mice and expression was assessed biweekly. Expression was still detectable at the end of the twelve week observation period.

Zhou et al. (Reference 7) describes the long-term expression of human factor IX by rabbit skin fibroblasts implanted into rabbits as autografts or allografts. Expression was shown to have been maintained for more than ten months at the time of preparation of the manuscript.

Scharfmann et al. (Reference 8) describes the long-term expression of foreign genes in mouse embryo fibroblast implants.

Shaw et al. (Reference 9) reported the adoptive transfer of T-cells to immunize mice. These T-cells were shown to delay the onset and reduce the severity of an experimentally induced autoimmune disease.

Brodeur et al., Kints et al., and Stewart et al. (References 10–12) each describe methods for injecting hybridomas into mice to produce large quantities of monoclonal antibodies.

Sugimoto and Hayashibara (Reference 13) describe a method for producing erythropoietin by transplanting human lymphoblastoid cells into a non-human warm-blooded animal body. The reference also states that the animal may be an adult, a newborn, a fetus, or an embryo.

Bronson et al. (Reference 14) describe a method for modifying embryonic stem cells to allow them to overexpress a protein of interest, and delivering the modified embryonic stem cells into an embryo to produce chimeric animals and transgenic animals expressing the protein of interest.

Since the claims are not directed to gene therapy, but generally to gene expression, in view of the established utility of *in vivo* gene expression, such as the utility demonstrated in the attached references, Applicants respectfully request that the rejection be withdrawn.

Applicants also point out that claim 77 is not directed to introducing the construct into a genome-containing cell *in vivo*. Accordingly, it is not clear why this claim has been included in the rejection. Claim 86 is directed to a method for producing an expression product

of an endogenous cellular gene *in vivo*. This claim has been amended to recite that the actual introduction of the vector is to a cell *in vitro*. Claim 87 is not directed to *in vivo* production either. In fact, the claim recites that the cell is cultured and expressed. Accordingly, it is not clear why this claim has been rejected on these grounds. Claim 98 is directed to a method for over-expressing an endogenous gene *in vivo*. This claim has been amended to recite that the introduction of the vector is to a cell *in vitro*. Claim 109 is not directed to expressing the protein *in vivo*. In fact, this claim is specifically directed to culturing cells in reduced-serum medium. Accordingly, it is not clear why this claim has been rejected on these grounds. The same lack of clarity applies to claim 110. Claim 113 is also directed to methods for culturing cells. Claim 116 is also directed to methods for culturing cells. Claim 223 is not directed to practicing the methods *in vivo*. Claim 225 is not directed to methods for expressing the gene *in vivo*. Likewise, claim 232 is not directed to expressing the gene *in vivo*, but is limited to culturing the cell.

In any event, regarding claims encompassing *in vivo* production of activated gene products, Applicants submit that these claims are not directed to direct introduction of a vector *in vivo* and that they are fully enabled for producing useful gene expression products *in vivo*.

On page 9 of the Office Action, the Examiner has rejected claims 104–106 as not enabled as follows:

> [the person of ordinary skill in the art] would not know whether the vector construct of the claimed method would integrate at the site of the double

> strand break because as emphasized by the specification, the method works due to random integration. There is nothing in the method steps or in the specification which would ensure that the vector. . .would have integrated at the site of DNA breaks. Therefore, an artisan would not even know whether the steps of DNA break [sic] would have any affect on the practicing of the method as recited.

Applicants respectfully disagree.

Applicants point out that the claims do not recite integration of the vector at the double strand break site. They recite introducing double strand breaks into genomic DNA at the same time or around the same time that the vector is integrated. To perform this step, is fully enabled. It simply requires introducing these breaks when the vector is integrated. Various techniques for introducing these breaks are provided in the specification. Accordingly, the specification provides adequate guidance for practicing the method. Moreover, Applicants have shown in their specification that the introduction of such breaks increases the efficiency of integration. Applicants need not understand the mechanism to enable the claims. All that is required is that the person of ordinary skill could make and use the method as claimed. In view of the above discussion, Applicants submit that the specification provides a way to introduce breaks and shows that the introduction of such breaks is indeed useful.

In view of the discussion and amendment to the claims, Applicants believe that they have addressed all of the Examiner's grounds for rejection under 35 U.S.C. § 112, first paragraph on the basis of non-enablement, and have succeeded in overcoming this rejection on

all grounds. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

D. The Rejection Under 35 U.S.C. § 112 Second Paragraph

On page 10 of the Office Action, claims 72, 73, 77–82, 85–89, 98, 105, 106, 109–123, 129, 157, 159, 161, 162, 165–167, 169–175, 177–183, 223–226, 232–259 have been rejected under 35 U.S.C. § 112, second paragraph, on the grounds that they are indefinite. Applicants respectfully traverse the rejection.

First, the Examiner indicates that claim 72 is incomplete because it is dependent on a cancelled claim. Applicants note that the Examiner included claim 72 as part of Group VII in the restriction requirement. Accordingly, clarification is requested. Claim 73 is likewise rejected as indefinite because it is dependent on claim 72. Applicants note that claims 71–74 were included in Group VII. Accordingly, clarification is requested.

On page 10 of the Office Action, claims 77 and 81 are rejected as indefinite on the grounds that the term "the construct" does not have antecedent basis because the base claim recites "a vector construct." Accordingly, claims 77 and 81 have been amended accordingly.

On page 10 of the Office Action, claims 77 and 113 have been rejected as indefinite on the grounds that the term "said endogenous gene" does not have antecedent basis. Specifically, the Examiner notes that the preamble recites "an endogenous cellular gene."

Accordingly, the claims have been amended so that the body of the claim conforms to the preamble.

On page 10 of the Office Action, claim 81 has also been rejected as indefinite on the grounds that the term "said construct" in line 2 does not have antecedent basis. Accordingly, the claim has been amended to provide a clear antecedent basis.

On page 10 of the Office Action, claims 85–87, 109, 110 and 116 have been rejected as indefinite on the grounds that the term "said gene" does not have antecedent basis. To more clearly identify the antecedent basis, which the Applicants believe is currently correct, the Applicants have inserted the term "endogenous" prior to the term "gene."

On page 10 of the Office Action, claim 89 has been rejected as indefinite on the grounds that it depends from claim 232, which is not a precedent claim. Accordingly, Applicants have amended claim 89 to delete reference to claim 232 and have introduced new dependent claim 260 that refers to claim 232.

On page 10 of the Office Action, claims 118 and 119 have been rejected as indefinite on the grounds that the term "genetic construct" does not have an antecedent basis. The term "genetic construct" is used for the first time as a first instance use of the term and the remainder of the uses of the term "genetic construct" in the claim refers to the first usage. Accordingly, Applicants submit that there is proper antecedent basis for all uses of the term

"genetic construct." Likewise, the use of "genetic construct" in claim 119 clearly refers back to the first instance use of the term "genetic construct" in claim 118.

On page 10 of the Office Action, claim 157 has been rejected as indefinite on the grounds that the term "said splice donor site" does not have an antecedent basis. Step (c) in this claim refers to the first instance use of a splice donor site. Accordingly, "said splice donor site" in the remainder of the claim would be understood as referring to the splice donor site in step (c) of the claim.

On page 11 of the Office Action, claims 159, 161, 162, 165–167, 169–17, 174, 175 and 177–179 have been rejected as being indefinite on the grounds that "said vector" or "the vector" does not have antecedent basis. Applicants assume that the Examiner refers to the fact that in the independent claim, the term "vector construct" is used. Accordingly, the claims at issue have been amended to recite the term "vector construct."

On page 11 of the Office Action, claim 181 has been rejected as indefinite on the grounds that it is unclear whether the term "an endogenous gene" is the same in the preamble and the body of the claim. Accordingly, the claim has been amended to provide antecedent basis for the second instance of the use of the phrase.

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On page 11 of the Office Action, claim 181 has been rejected as indefinite on the grounds that the terms "the host cell," "said cells," and "said selected cells" do not have

antecedent basis. The claims have been amended to remove the term "host." Step (c) has been amended to recite that this step produces selected cells. With respect, however, to the objection to the term "said cells," Applicants assert that there is no basis for this objection since the term clearly relates back to the cells recited in step (a).

On page 11 of the Office Action, claim 223 has been rejected on the grounds that the phrase "suitable for activating an endogenous gene" is unclear. Applicants respectfully disagree. Applicants respectfully submit that the person of ordinary skill in the art would have recognized the metes and bounds of this limitation as referring to a vector that is capable of activating an endogenous gene. This language is routinely used in issued claims from the U.S. Patent and Trademark Office. Accordingly, Applicants believe that the claim meets the requirements of 35 U.S.C. § 112, second paragraph.

On page 11 of the Office Action, claim 232 has been rejected on the grounds that it is unclear whether the endogenous gene in the preamble of the claim is the same gene recited in the body of the claim in step (c). The claim has been amended in step (c) to more clearly point out that over-expression is of the endogenous cellular gene in the preamble.

On page 11 of the Office Action, claim 232 is further rejected as indefinite on the grounds that the phrase "said isolated and cloned cell" does not have antecedent basis. The claim, accordingly, has been amended to delete this phrase.

On page 11 of the Office Action, claim 224 has been rejected as indefinite on the grounds that the term "said host cell" does not have antecedent basis. Claim 224 refers to claim 223. Claim 223 refers to a host cell in step (a). Accordingly, Applicants submit that the term has proper antecedent basis.

On page 11 of the Office Action, the Examiner indicates that claim 234 is indefinite because it is incomplete and does not have a period at the end. Applicants point that Applicants' attorney erroneously overlaid a Certificate of Transmission over claim 234, thereby deleting the last three lines of text. Accordingly, Applicants resubmit this claim in its entirety, but which may be further amended in response to other issues contained herein.

On page 11 of the Office Action, claims 234–241, 243–245, 247 and 257 have been rejected on the grounds that they are indefinite because the term "said construct" does not have antecedent basis. Applicants have amended the claims.

On page 11 of the Office Action, claim 236 has been rejected on the grounds that it is indefinite by reciting the term "said exon does not contain a selectable marker" because a selectable marker is a protein and an exon is a DNA sequence. The claim, accordingly, has been amended to recite that the exon does not contain sequences encoding a selectable marker.

On page 11 of the Office Action, claims 58, 59 and 157 are rejected on the grounds that they are indefinite in recitation of a vector construct containing an amplifiable

marker or selectable marker since these are proteins and the vector is nucleic acid. Accordingly, the claims have been amended to recite that the vector contains sequences encoding these proteins.

On page 11 of the Office Action, claim 246 has been rejected on the grounds that it is indefinite in reciting the term "the vector construct" without antecedent basis. Applicants respectfully submit that the term has antecedent basis because in line 1 of the claim, the term "vector construct" is clearly recited.

On page 11 of the Office Action, claims 249 and 250 have been rejected as indefinite on the grounds that the term "the construct" does not have antecedent basis. Applicants have amended the claim.

On page 12 of the Office Action, claims 256–258 have been rejected as indefinite on the grounds that the term "said cell" does not have antecedent basis. In claim 256, the term clearly refers back to the precedent use of the term "cell." Applicants do not understand the basis for the Examiner's objection. Same applies to claim 257, which clearly refers back to the first use of the term "cell." Similarly, the use of the term "said cell" in claim 258 clearly refers back to the first instance of the term "cell."

On page 12 of the Office Action, claim 257 has been rejected on the grounds that the term "said eukaryotic cell" does not have antecedent basis. Applicants point to the first use of the term "eukaryotic cell" in claim 257 on line 2. Accordingly, there is clear antecedent basis.

On page 12 of the Office Action, claim 159 has been rejected on the grounds that the term "transposition signal" is unclear. Applicants respectfully submit that this is a term of art that would clearly have been understood by the person of ordinary skill in the art to refer to those sequences that are necessary for conferring transposition function. The Examiner is directed to pages 108–109 of the present specification for disclosure of transposition signals. It would have been understood that such signals are those that confer the transposition function to a vector sequence.

On the basis of the above discussion and amendments, Applicants believe that they have addressed all of the Examiner's objections under 35 U.S.C. § 112, second paragraph, and have overcome each of the grounds for rejection. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

E. The Rejection Under 35 U.S.C. § 102(b)

On page 12 of the Office Action, claims 58, 64, 69, 74, 76, 234–241, 243 and 247 have been rejected under 35 U.S.C. § 102(b) on the grounds that they are anticipated by Reilly *et al.* (*DNA and Cell Biology*, 9:535–542 (1990)), herein "Reilly." Applicants respectfully traverse the rejection.

The Examiner relies on Reilly for teaching a vector containing the T7 promoter linked to exon I of human growth hormone. The reliance is erroneous. The Reilly vector would never contain an unpaired human growth hormone splice donor.

Reilly provides the following two vectors: pBSD, shown in Figure 1B, and pBSA, shown in Figure 1C. pBSD is designed to identify splice acceptor sites in a cloned genomic fragment. It contains an exon with an unpaired splice donor site, both derived from *adenovirus*. A multiple cloning site is located downstream from this exon-splice donor. A genomic fragment can be cloned into the multiple cloning site. A transcript is initiated from the T7 promoter that proceeds through the vector sequences into the genomic fragment cloned into the multiple cloning site. If a splice acceptor site is present in the genomic fragment, the vector should allow splicing to occur from the adenovirus splice donor onto the splice acceptor.

Vector pBSA is designed to identify splice donor sequences in a cloned genomic fragment. Thus, the vector contains an adenovirus exon that has a splice acceptor site. The multiple cloning site is located upstream from this exon. A genomic fragment can be cloned into the multiple cloning site. If this genomic fragment contains a splice donor sequence, splicing will occur from the splice donor site on the genomic fragment to the splice acceptor site on the vector.

Reilly tested the splicing capability of pBSD. A human growth hormone exon with a *splice acceptor* site was inserted into the multiple cloning site. Accordingly, in pBSD, the T7 promoter was not linked to an unpaired human growth hormone exon-splice donor sequence.

In pBSA, a human growth hormone exon-splice donor sequence was inserted into the multiple cloning site to test the splice acceptor capability of the vector. Note, however, that there is already an adenovirus splice acceptor on the vector. Accordingly, when the human growth hormone exon-splice donor sequence is introduced into pBSA, it is *paired* with a splice acceptor sequence (the adenovirus splice acceptor sequence on the vector).

Thus, contrary to the Examiner's exertion, the T7 promoter is never linked to an unpaired splice donor sequence of human growth hormone.

Accordingly, to the extent that the rejection is based on the assumption that the vector contains an unpaired human growth hormone splice donor and exon sequences operably linked to a T7 promoter, the rejection is in error and should be withdrawn.

Claims 234–241, 243 and 247 have been amended to limit the promoter to promoters other than that found on the pBluescript vector. Accordingly, the claims, as amended, would not have been anticipated by Reilly.

Moreover, there is no suggestion to create a vector from other than pBluescript or to modify the pBluescript vector, such as by changing the promoter. Reilly specifically constructs the splicing vector from pBluescript, a commercially-available plasmid. The T7 promoter is part of the vector design. It functions according to the purpose of Reilly. The Reilly vector is designed to detect chimeric transcripts that are produced by splicing. In the discussion, first line, Reilly indicates that "processing the human growth hormone chimeric transcripts in HeLa nuclear extracts for two hours was sufficient to enable identification of the spliced exons. . ." Since the vector described by Reilly operates sufficiently for splicing, there would have been no motivation to modify the vector of Reilly, for example, by replacing the T7 promoter with a cellular promoter or a eukaryotic viral promoter. In fact, since *in vitro* transcription using cellular and eukaryotic viral promoters is more complicated and much less efficient, substitution of these promoters would be detrimental. Accordingly, the claims as amended would not have been obvious over Reilly.

The rejection is also based on the assumption that lacZ is an amplifiable marker. Applicants respectfully submit that this is incorrect. The term "amplifiable marker" is a term of art which would have been well-known to the person of ordinary skill in the art. The term refers to a sequence that is amplifiable *per se* in response to selective pressure. See pages 40–41, 69–70, and 98–99 of Applicants' present specification. When the marker is integrated in a cellular genome, nucleic acid sequences flanking the amplifiable marker sequences become amplified along with the marker in response to the selective pressure asserted on the marker.

Gene amplification has been observed since the early 1950s and has been studied and documented for at least the last two decades. The initial observation was that stepwise selection in increasing concentrations of methotrexate resulted in animal cells with increased levels of the enzyme dihydrofolate reductase (DHFR). The increased level of the enzyme was the result of an increase in the DHFR gene copy number. Accordingly, in cells subjected to growth in methotrexate, a population of cells containing amplified copies of the DHFR gene outgrow the cell population. The amount of gene amplification, in general, is proportional to the level of gene expression. Therefore, by applying the appropriate selection pressure, the number of amplified copies of the target gene can be controlled.

The most widely used amplifiable marker is DHFR. However, many other genes have been used for this purpose. These include metallothionein, CAD, adenosine deaminase, adenylate deaminase, UMP synthetase, IMP 5′-dehydrogenase, xanthine-guanine phosphoribosyltransferase, mutant HGPRTase or mutant thymidine kinase, thymidylate synthetase, p-glycoprotein 170, ribonucleotide reductase, glutamine synthetase, asparagine synthetase, arginosuccinate synthetase, ornithine decarboxylase, HMG-CoA reductase, *N*-acetylglucosaminyl transferase, theronyl-tRNA synthetase, and Na^+, K^+-ATPase.

Amplifiable markers have been useful to co-amplify genes that cannot be amplified by direct selection. Thus, co-amplification of desired genes has been used to produce cell lines expressing high levels of the desired protein which is co-amplified along with the

amplifiable marker gene. An amplifiable marker, when integrated in a cellular genome, will, therefore, cause co-amplification of genomic DNA in the proximity of the amplifiable marker.

Amplifiable markers are generally selectable markers that may be dominant or recessive. The art, however, distinguishes amplifiable markers from other selectable markers, such as bacterial markers, for example, those conferring resistance to neomycin, kanamycin, and similar compounds in bacterial cells. Whereas these bacterial genes can function in mammalian cells to confer resistance to G418 and hygromycin, respectively, these selection markers are not amplifiable. They are often used on expression vectors to select the initial transformed cells. Then, the amplifiable marker is used to amplify copies of the target gene in the cells selected by means of the non-amplifiable selectable marker. Accordingly, initial transformants are selected by means of a non-amplifiable selectable marker and these transformants are then subjected to selection pressures that result in expression and amplification of the amplifiable marker (and any genes co-amplified with this marker).

With respect to these points, Applicants provide a review article (Exhibit E1) entitled "Selection and Co-Amplification of Heterologous Genes in Mammalian Cells." This reference is submitted to show how the art understood the term "amplifiable marker" and to show that the art distinguished between markers that are merely selectable and markers that are selectable and/or amplifiable, and to show how amplifiable markers can be used to co-amplify desired genes that cannot be amplified alone.

First, Applicants point out that the article states that not all genes can be amplified by applying selective pressure. For many genes, direct selection methods are not available. The article states that in these cases, it is possible to introduce the gene of interest with a selectable and amplifiable marker and, subsequently, to select for amplification of the marker gene to generate cells that have co-amplified the desired gene. See the paragraph spanning pages 537 and 538.

Table 1, on page 545, shows dominant and recessive amplifiable genetic markers.

On page 548, the section headed "Co-Transfection with Another Dominant Selectable Marker" shows that the art distinguishes between selectable markers and selectable amplifiable markers. The section states that initial transformants can be isolated using a selectable marker, such as neo. This marker, as explained in the text, is co-amplified because of the presence of the amplifiable marker DHFR. In this case, a gene of interest is also co-amplified along with the DHFR and neo genes. The article, however, refers to DHFR as the amplifiable marker. Indeed, in the paragraph spanning pages 558 and 559, the reference states that neither the neo gene nor another bacterial selectable marker gene, hyg, has been demonstrated to be amplifiable.

Applicants also submit several U.S. patents containing claims that recite the term "amplifiable marker" *without further limitation on this term.* Applicants reviewed the specifications to ascertain if and how the patentees defined the term in the specification.

Applicants' position is that the term is a term of art and, therefore, would not have been used in the specification in a manner other than in the art-recognized manner. Thus, Applicants reviewed the specification to ascertain whether the meaning in the specification deviated from the meaning in the art. In fact, the term was not defined; the disclosure merely explained how such markers function. Accordingly, Applicants submit that these patents reflect the use of the term as understood by the art.

U.S. Patent No. 5,733,746 (Exhibit E2) contains two claims of relevance. Claim 3 is directed to a DNA construct "further comprising a selectable marker gene." Claim 4 is directed to a DNA construct "further comprising an amplifiable marker gene." The fact that these claims distinguish two marker types, without further limitation, shows that these terms were considered different by the U.S. Patent and Trademark Office and would have been understood, in and of themselves, as terms of art. The specification refers to selectable or amplifiable markers. In column 5, lines 44–53, the text states:

> "the gene can be further amplified by the inclusion of an amplifiable selectable marker gene which has the property that cells containing the amplified copies of the selectable marker gene can be selected for by culturing the cells in the presence of the appropriate selectable agent. The activated gene is amplified in tandem with the amplifiable selectable marker gene."

The use of the term as above, without specific definition, shows that the art would have understood the term to refer to marker genes functioning as the Applicants have discussed above.

In column 11, lines 5–7. the text is as follows:

"As described herein, there are frequently additional construct components, such as a selectable and/or amplifiable marker."

In column 13, the Examiner is directed to the section entitled "Marker Genes for Selection and Amplification." The text contains, in pertinent part, the following:

"The identification of the targeting event can be facilitated by the use of one or more selectable marker genes. . .

. . .cells containing. . .transcription units. . .may be isolated by including within the targeting DNA construct, an amplifiable marker gene, which has the property that cells containing multiple copies of the selectable marker gene, can be selected for by culturing the cells in the presence of an appropriate selectable agent. The novel transcription unit will be amplified in tandem with the amplified selectable marker gene, allowing the production of very high levels of the desired protein. Amplifiable marker genes and their use are described in U.S. Patent Application Nos. 08/243,391, 07/985,548 and PCT/US93/11704.

In one embodiment, the positive selectable marker neo is used. . .to select for cells which have stably incorporated the DNA of targeting construct, and the mouse DHFR. . .gene is used to subsequently amplify the novel transcription unit. . ."

Here, again, the text uses the term "amplifiable marker" to refer to that gene used to amplify a desired transcription unit, in distinction to the mere selection marker, neo, used to identify cells that have incorporated the DNA.

The text refers to U.S. Patent Application No. 08/243,391. This application does not contain claims directed to an amplifiable marker, but uses the term in the specification. In column 12, under the section labeled "Selectable Markers and Amplification," the text discusses the identification of a targeting event that can be facilitated by the use of one or more selectable marker genes. The text indicates that a variety of selectable markers can be incorporated into the cells. Selectable marker genes are indicated to include "neo, gpt, DHFR, ada, pac, hyg, cad, gs, mdr-1, and hisD. The text also indicates that the selectable phenotype "makes it possible to identify and isolate recipient cells." The text then goes on to specifically distinguish amplifiable genes as follows:

> "Amplifiable genes encoding selectable markers (e.g., ada, gs, DHFR and the multi-functional CAD gene) have the *added characteristic that they enable the selection of cells containing amplified copies of the selectable marker inserted into the genome.* This feature provides a mechanism for significantly increasing the copy number of an adjacent or linked gene for which amplification is desirable. Mutated versions of these sequences showing improved selection properties and other amplifiable sequences can also be used."

Accordingly, the reference distinguishes between selectable and amplifiable sequences. (Emphasis added).

U.S. Patent No. 5,612,213 (Exhibit E3) contains claims reciting the term "amplifiable marker" without further definition or limitation. In column 1, in Background of the Invention, the text is as follows:

> "Gene amplification is a strategy which has been broadly applied to increase protein production in mammalian cells. A transcription unit. . .encoding a protein of interest is normally linked covalently to an amplifiable marker. The transcription unit and marker are then co-transfected into appropriate cells, followed by selection and amplification as discussed below."

Applicants point out that this text is submitted under the section labeled "Prior Art." The text then goes on, in column 1, to discuss gene amplification as a widely used transfection and amplification strategy. It explains how an amplifiable marker functions. Accordingly, Applicants submit that this text demonstrates that gene amplification, a widely-known biological process, would have been understood to occur by means of an amplifiable marker and that the term "amplifiable marker" would have been understood to be that which facilitates gene amplification based on selection available against that marker.

In the Summary of the Invention, column 3, lines 29–45, the term is, again, used without further explanation, indicating that a preferred amplifiable marker is DHFR, although other markers, such as gs and mdr, could also be used.

U.S. Patent No. 5,561,053 (Exhibit B) contains claims directed to amplifiable genes without further limitation. Claim 4 is directed to a DNA construct "wherein the selectable gene is an amplifiable gene." Claim 5 is directed to the "DNA construct of claim 4, wherein the amplifiable gene is DHFR." Claim 8 is directed to a vector, "wherein a selectable gene of the DNA construct is an amplifiable gene." In the specification, in the fourth paragraph, under the heading "Description of the Related Art," the specification discusses specifically a selectable gene that encodes a selectable marker. It is indicated that selectable genes commonly used with eukaryotic cells include genes for APH, hyg, DHFR, tk, neo, puro, gs, and aps. In that same section, however, the text specifically discusses amplifiable markers. In paragraph 11, under that section, the reference discusses a method for obtaining a high gene copy number that "involves gene amplification in the host cell." It indicates that gene amplification may be induced or selected for by exposing host cells to appropriate selective pressure. It states:

> "In many cases, it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the co-transfected cells to sequentially increasing concentrations of a selected agent. Typically, the product gene will be co-amplified with the marker gene under such conditions.
>
> The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate."

The section goes on to suggest that host cells could also be co-transfected with the desired gene, a DHFR gene, and a dominant selectable gene, such as neo. It is indicated that transfections are

identified by culturing the cells first in neo, and then the transfections are selected for amplification of the DHFR gene by exposure to methotrexate.

The above text indicates, as in the preceding cases, that an amplifiable marker would have been understood in the art to be that sequence which can be selected for and, in doing so, produces amplified copies of itself in the cell. This is in distinction to a merely selectable marker which allows only selection of a cell containing the marker, and not for amplification of the marker.

Applicants also point out that the U.S. patents cited were issued to three independent patentees. Accordingly, Applicants submit that the term is not one merely propagated by a specific laboratory or applicant.

In the present case, however, the plasmid is not amplified by means of the lacZ gene. The lacZ gene is merely replicated along with the plasmid. If the lacZ gene were deleted from the plasmid, the plasmid would still be a multicopy plasmid.

Accordingly, to the extent that the rejection is based upon the vector disclosed in Reilly as containing an amplifiable marker, the rejection is in error and should be withdrawn. Based on the above discussion, Applicants submit that the pending claims are not anticipated by Reilly. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

F. The Rejection Under 35 U.S.C. § 102(e)

On page 12 of the Office Action, claims 59, 69, 71, 73, 74, 76, 225 and 226 have been rejected under 35 U.S.C. § 102(e) as being anticipated by Treco *et al.* (U.S. Patent No. 5,641,670), herein "Treco." Applicants respectfully traverse the rejection.

Independent claim 225 recites the limitation that the transcriptional regulatory sequence is operably linked to a gene. The Treco vectors, however, do not contain this limitation. Accordingly, Treco does not anticipate claim 225.

Further, the constructs in Treco contain targeting sequences to promote homologous recombination. Applicants' vectors are distinguished over those disclosed in Treco in that the Applicants' claimed vectors do not contain any targeting sequences. Presently, pending claim 59 reflects this limitation. Targeting sequences are discussed in Applicants' specification on pages 30–31.

In view of the amendment and above discussion, Applicants submit that the claims are not anticipated by Treco. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

G. The Rejection Under 35 U.S.C. § 102(b) / 103(a)

On page 13 of the Office Action, claims 77, 78, 80–82, 87–89, 109, 114, 116–123, 129, 157, 159, 161, 162, 164–167, 169–175, 177–183, 223, 224, 232 and 233 have been

rejected under 35 U.S.C. § 102(b) as being anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Treco as evidenced by Capecchi *et al.* (*Scientific American*, pages 52–59 (March 1994)), herein "Capecchi." Applicants respectfully traverse the rejection.

First, Applicants submit that references have been improperly combined for the purposes of anticipation in the instant rejection. While additional references may be used to interpret an allegedly anticipating reference and may be used to shed light on what it would have meant to the person of ordinary skill in the art, in the present case, Capecchi is not being used to interpret Treco or to show how the person of ordinary skill in the art would have understood Treco. Capecchi provides additional information on the events alleged to occur using the Treco methods. Applicants point out that, in fact, the information provided by Capecchi is actually provided in the Treco reference itself. See column 21, lines 45–52.

The above argument notwithstanding, Applicants refer to the discussion above regarding the fact that Treco does not anticipate vectors without targeting sequences and accordingly, uses of such vectors.

Moreover, it would not have been obvious from the references to make or use a vector lacking targeting sequences. This is because the fundamental design of the vectors and their use require targeting sequences. The '670 patent teaches that activation can only be achieved by homologous recombination. In column 36, lines 52-53, the reference states that cells in which the transfecting DNA integrates randomly into the genome cannot produce EPO

(the target gene). The '670 patent, therefore, actually teaches away from non-homologous recombination to activate endogenous genes and, therefore, teaches away from making and/or using vectors that lack targeting sequences to activate endogenous genes.

The Examiner cites Capecchi as showing that most of the insertion events with the targeted vectors are random insertion events. The '670 patent, in fact, also taught that the majority of integration events were not targeted events. In column 21, lines 45-52, the '670 patent indicates that homologous recombination events are masked by a vast excess of events in which integration is by non-homologous recombination. But this would not have made it obvious to make or use vectors without targeting sequences. Cells in which these non-targeted events occurred were not retained. The '670 patent teaches retaining only cells in which a homologous recombination event has occurred. Therefore, there would have been no motivation to activate an endogenous gene by non-homologous recombination. Accordingly, there would have been no motivation to make or use vectors without target sequences to activate genes.

On page 13 of the Office Action, the Examiner states that the claimed vectors and the Treco vectors are the same. This is incorrect. The Treco vectors contain a homologous targeting sequence while the vectors of claims 58 and 59 do not.

On page 14 of the Office Action, the Examiner indicates that it would have been obvious to screen non-homologously recombinant cells for gene activation. However, Treco specifically screened only homologously recombinant cells and discarded the remainder because

Treco believed that only homologous recombination events would activate a gene of interest. Accordingly, Treco neither disclosed or suggested screening non-homologously recombinant cells.

On page 14 of the Office Action, the Examiner further states that non-homologous recombination events with the Treco vectors will result in random activation of non-targeted genes. No evidence of activation, however, was shown in either the '670 patent or by Capecchi. And, as discussed above, the '670 patent states that only homologous recombination events would be sufficient to activate EPO. Accordingly, even if such an activation event had accidentally occurred, it would have been unrecognized and unappreciated by the person of ordinary skill in the art as it was unrecognized and unappreciated in the cited reference. The fact that such events could be achieved was shown for the first time in Applicants' own disclosure.

Accordingly, the cited art neither anticipates nor renders obvious any of the Applicants' claims. Reconsideration and withdrawal of the rejection on these grounds is, therefore, respectfully requested.

H. The Rejection Under 35 U.S.C. § 102(b) / 103(a)

On page 14 of the Office Action, claims 109–113 and 115 have been rejected under 35 U.S.C. § 102(b) as anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Joshi (*Medical Hypothesis*, 36:242–245 (1991)), herein "Joshi." Applicants respectfully traverse the rejection.

First, Applicants note that independent claims 109 and 110 are directed to culturing the cells in reduced serum medium. Joshi neither teaches nor suggests this step. Further, the Examiner has provided no evidence or rationale showing that Joshi discloses or suggests this particular step. On page 15 of the Office Action, the Examiner generally refers to "the most important step in the claimed method" as being step (b), which is integrating the vector into the genome by non-homologous recombination. The Examiner states that this is "the main teaching of Joshi." He does not analyze the other steps other than to state that the other steps "are art-recognized conventional steps of cell culture and protein screening."

With respect to the requirements for anticipation, Applicants submit that even if a step is conventional, if the step is missing in the cited reference, the reference cannot anticipate the claims. Because Joshi does not disclose growing cells in reduced serum medium, Joshi cannot anticipate these claims.

With respect to the requirements for obviousness, the art must contain some suggestion to practice the claimed invention. There is no disclosure in Joshi, however, that would motivate the person of ordinary skill in the art to culture the cells in reduced serum medium. Further, it is incumbent upon the Examiner to point out the suggestion. But, the Examiner has provided no reason why it would have been desirable to grow the cells in reduced serum medium. Further, the Examiner has not provided a reason to support his position that it

would have been obvious to screen the cell-conditioned medium for the presence of the expression product.

Joshi states that the report describes "the potential use of such retroviral vectors to create cell lines expressing 'silent' genes and to isolate genomic and cDNA clones." Column 2, page 243. The proposed strategy for cloning such genes in mammalian cells is shown in Figure 1. Nothing in this flowchart suggests using reduced serum medium. Studies are done using viral RNA, cDNA, or genomic DNA. Translational studies are performed with RNA isolated from viral particles. Gene function is studied following cDNA cloning of the gene. Gene function is also studied using isolated wild-type genomic DNA or by means of cloned flanking DNA sequences.

The flowchart also indicates "further applications" for clones with a specific phenotype. However, the flowchart does not define such applications. Column 2, page 243, indicates that mutants expressing an activated phenotype could be used "for further research purposes as well as for genomic or cDNA cloning of the insertionally activated gene responsible for this new phenotype." Column 2, page 244, states that "once a mutant expressing the desired phenotype is screened, it could be used for further applications (i.e., enzyme kinetics, drug testing) as well as for genomic or cDNA cloning of the insertionally-activated gene responsible for the phenotype." Accordingly, none of this text suggests growing cells in reduced serum medium.

In summary, even if growing and screening cells in reduced serum is conventional, there still must be a suggestion in the art to do so. The cited art does not provide the motivation. The Examiner fails to indicate a reason.

If this rejection is maintained, Applicants respectfully request that the Examiner cite relevant sections in the reference that would provide the motivation. If this citation is not set forth, Applicants request that the Examiner explain how such steps would have been obvious to perform with the Joshi process.

In view of the argument and evidence above, Joshi neither anticipates nor suggests the claimed methods.

The Examiner has also rejected claim 113 on the same grounds, i.e., that step (f) is "an art-recognized conventional step of cell culture and protein screening." Applicants respectfully disagree.

There is nothing in Joshi that discloses or provides the motivation to produce a cell mass equivalent of at least 10 liters of cells at 10^4 cells/ml. Joshi discusses protein production for further characterization, not in commercial amounts. For this reason alone, Joshi does not disclose or suggest this limitation.

Further, however, Joshi specifically recites that the proteins are expressed from cDNA clones, not directly from the endogenous activated gene. Specifically, on page 245, Joshi discusses **RNA** isolation from retroviral particles containing the 3′ flanking sequences (i.e., "activated" gene) and the use of the viral RNA for **cDNA** cloning. The reference then discusses the use of the cDNA clones as follows "these clones would be characterized and used for transfection of the parental (X^-) cell line. The full-length clone should give rise to a positive phenotype. **This cDNA clone could then be expressed in large amounts, and the resulting protein(s) purified and further characterized."** (Emphasis added).

This is the only text that discusses the production of proteins for any reason. These proteins are used for analytical purposes, and not commercial purposes. Accordingly, there would have been no suggestion to produce such a high cell mass from which to isolate the expression product. Further, since the expression product is produced from the cDNA clone, there is no disclosure or suggestion to produce the expression product and isolated directly from cells containing an activated gene.

For these reasons, claim 113 is neither anticipated by nor obvious over Joshi.

The Examiner also rejects claim 115 over Joshi on the grounds that Joshi teaches a vector that "has a 5′ sequence which has the packaging signal and splice donor site (see Figure 2 and description in paragraph 1 on page 244)." Applicants respectfully disagree. Joshi did not use an activation vector that contains a splice donor sequence. The activation vectors were used

to produce transcripts that were initiated at the 3′ LTR. In order to provide packagable retrovirus particles, the packaging sequence was inserted in the 5′ LTR between the U3 and U5 sequences. This is shown in a parental vector in the top diagram of Figure 2. In this vector, the splice donor sequence was deleted. This deletion vector was then used make the indicated vectors in Figure 2, which were then used as the activation vectors. The Figure indicates that in the parent vector, the U5–pBS–SD sequences were deleted and that in the subsequent vectors that the U5-pBS was inserted within the packaging region at three different locations. Accordingly, the reference teaches that vectors do not, in fact, contain a splice donor site when used as the activation vector.

For the reasons discussed above, the claims are neither anticipated by nor obvious over Joshi. Reconsideration and withdrawal of the rejection on the stated grounds is, therefore, respectfully requested.

I. The Rejection Under 35 U.S.C. § 102(b)

On page 15 of the Office Action, claims 59, 69, 74, 76, 225 and 226 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Benedetti *et al.* (*Nucleic Acids Research*, 19:1925–1931 (1991)), herein "Benedetti." Applicants respectfully traverse the rejection.

The Examiner relies on Benedetti for teaching a transcriptional regulatory sequence, amplifiable marker, and viral origin of replication. The neo gene is shown to increase an episomal plasmid copy number. The reference does not disclose or suggest that the neo gene

has the properties of an amplifiable marker in that it would be expected to cause co-amplification of genomic sequences in proximity to the site of marker integration. In fact, with all the extensive research performed in the field of gene amplification, it has never been demonstrated that the neo gene can be expected to co-amplify a linked nucleic acid sequence when integrated in the genome. Note that this gene is not listed as an amplifiable marker in any of the attached references, whereas it is recognized in these references as a selectable marker. Kaufman (Exhibit E1), an expert in the field of gene amplification, in fact, states that the neo gene is not considered in the art to constitute an amplifiable marker. See Applicants' discussion on pages 56–65 herein and Kaufman, paragraph spanning pages 558–559. Kaufman discusses co-amplification of DNA sequences integrated in the cellular genome on pages 541–542.

In view of the above discussion, Applicant respectfully submits that the claims are not anticipated by Benedetti. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

J. The Rejection Under 35 U.S.C. § 102(b)

On page 15 of the Office Action, claims 234–259 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Zambrowicz *et al.* (*Nature*, 392:608–611 (1998)), herein "Zambrowicz." Applicants respectfully traverse the rejection.

First, Applicants point out that Zambrowicz teaches an exon that contains a selectable marker. In contrast, the claims are directed to vectors in which the exon does not

comprise a selectable marker. Accordingly, for this reason alone, the reference does not anticipate the claims.

Further, Applicants' claims are supported by the specification in Applicants' earliest priority document, U.S. Application No. 08/941,223, filed September 26, 1997. Zambrowicz published in 1998. Accordingly, Zambrowicz cannot anticipate the claims.

On page 2 of the Office Action, the Examiner asserts that Applicants' specifications do not disclose the limitations of claims 234–259. Applicants disagree as follows.

The Examiner is directed to Section IIIB above. Applicants submit that they have provided evidence and argument to overcome this rejection. Since Applicants' disclosure for these claims antedates Zambrowicz, Applicants respectfully submit that the reference does not anticipate the claims. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

K. The Rejection Under 35 U.S.C. § 102(e)

On page 16 of the Office Action, claims 234–259 have been rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,080,576, herein "the '576 patent." Applicants respectfully traverse the rejection.

The Examiner has taken the position that the current claims are not supported by the Applicants' earliest priority application and that, therefore, these claims are anticipated by the '576 patent, filed on April 8, 1998 (based on provisional Application No. 60/079,729, filed March 27, 1998). Applicants have discussed the issue of adequate support for the claims in Applicants' earliest priority application. Applicants have shown how the claims are supported by disclosure in this application. Accordingly, Applicants' claims would not be anticipated by the '576 patent, which was filed later than Applicants' priority application.

In view of the above, Applicants request reconsideration and withdrawal of the rejection.

L. The Rejection Under 35 U.S.C. § 103(a)

On page 17 of the Office Action, claims 65–68 and 71 have been rejected under 35 U.S.C. § 103(a) on the grounds that they are unpatentable over Reilly in view of Kaufman (*Short Protocols in Molecular Biology*, 1658–1660 (1995)), herein "Kaufman" and Dominski *et al.* (*Mol. Cell Biol.*, 11:6075–6083, (1991)), herein "Dominski." Applicants respectfully traverse the rejection.

The Examiner indicates that Reilly has been relied upon "[as] summarized previously (paragraph 15)." Applicants have already discussed why Reilly cannot be relied upon for teaching the lacZ gene as an amplifiable marker. Accordingly, Reilly fails as a primary reference.

The Examiner relies on Kaufman as a secondary reference. The Examiner relies on Kaufman to provide the motivation to replace the T7 promoter with a different promoter, such as a viral promoter or inducible promoter. Applicants respectfully disagree that the person of ordinary skill in the art would have been motivated by Kaufman to replace the T7 promoter.

Kaufman is directed to vectors for producing protein. Reilly is directed to vectors for splicing, not for producing protein. Accordingly, the teachings of Kaufman do not apply to the vectors of Reilly.

The Examiner states that Kaufman compares expression levels from different promoters. But, contrary to the Examiner's assertion, Kaufman does not disclose expressing protein by means of a viral promoter.

Further, Kaufman's suggestion to use an inducible promoter would not apply to the Reilly vectors. The use of an inducible promoter is discussed under the heading "Choice of Expression System." Kaufman indicates that if the protein is cytotoxic, then high-level expression could be approached through an inducible promoter system. Cytotoxicity would not be an issue with the Reilly vector because (1) it is used in cell-free extracts and (2) it does not produce protein. Therefore, there would have been no motivation to provide an inducible promoter on the Reilly vector.

In summary, Kaufman fails to motivate the person of ordinary skill in the art to replace the T7 promoter with any other promoter, much less, with the claimed promoters (viral, cytomegalovirus, or inducible), to be used in the Reilly splicing system.

Kaufman is further relied upon to motivate the person of ordinary skill in the art to stably integrate the vector in the genome. The Examiner's rationale is that a cell line would be produced that expressed a target coding sequence constitutively. The Examiner argues as follows: "furthermore, an artisan would have been motivated to select cells that would have integrated the vector [sic] in their genome because stable transfectants would have helped in producing cell lines that would have expressed a target coding sequence constitutively." Applicants disagree. First, there is no target coding sequence in the Reilly vector. The Reilly vector is designed to produce a transcript and not a protein. Accordingly, there would have been no motivation for "producing cell lines that would have expressed a target coding sequence constitutively." Accordingly, there would have been no motivation to stably integrate the Reilly vector for producing protein.

Moreover, as the Examiner recognizes, Kaufman teaches stable integration to produce milligram amounts of protein (Kaufman, 16–58). As discussed, the Reilly vector is not designed to produce any protein, much less milligram amounts. Accordingly, there would have been no motivation to stably transfect the Reilly vector by integrating it into the genome.

The Examiner also relies on Dominski for teaching the "role of exon length in splice site selection for pre-mRNA processing" and for teaching subcloning of "the constructs for *in vitro* splicing in plasmids that have CMV promoter for *in vivo* studies." The Examiner's rationale appears to be stated on page 18 of the Office Action as follows "an artisan would have been motivated to use recited promoters because this would have allowed testing the splicing efficiency *in vivo* in a cell (as done by Dominski et al.) and a promoter of choice would be used, keeping in mind the conventional wisdom that the expression level of foreign genes under the control of different promoter varies greatly based on the cell type used as discussed by Kaufman (see section on Choice of Expression System on page 16-60)." Applicants respectfully disagree.

The Examiner appears to take the position that (1) because Dominski tests a splicing vector in a cell, this provides the motivation to introduce the Reilly vector into a cell and (2) if the Reilly vector is in a cell, Kaufman suggests modifying the promoter.

However, Applicants submit that Dominski would not have provided the motivation to introduce the Reilly vector into a cell. This is because the Reilly vector was sufficient for its intended purpose. Note that in the discussion, first line, Reilly indicates that "processing the human growth hormone chimeric transcripts in HeLa nuclear extracts for two hours was sufficient to enable identification of the spliced exons. . ." Since the vectors were designed for this purpose and the system designed by Reilly operates sufficiently for its intended purpose, Dominski would not have motivated introducing the Reilly vectors into a cell.

Applicants further point out that the rejection also fails because Reilly cannot be relied upon for teaching an amplifiable marker. Applicants point out, however, that the Examiner has not relied on any of the secondary references to motivate the placement of an amplifiable marker on the Reilly vector. Indeed, no motivation to provide an amplifiable marker on the Reilly vector is provided by any of the secondary references.

Applicants point out that Kaufman does discuss amplification of protein expression vectors (page 16–59). Kaufman suggests co-transfecting an amplifiable marker when the protein expression sequence is stably transfected (integrated). But, since Reilly does not produce protein, there is no motivation either to stably transfect (integrate) the Reilly vector. Since there is no motivation to integrate, there would be no motivation to amplify either.

Furthermore, as discussed above, there was no motivation even to put the Reilly vectors in an intact cell. The Reilly vectors were used to synthesize chimeric RNA transcripts in *in vitro* splicing extracts. Reilly indicates that the extracts were "sufficient to enable identification of the spliced exons. . ." Since the system operates sufficiently, Reilly would not have suggested introducing the vectors into a cell. Since there was no motivation to introduce the vector into a cell, there would have been no motivation to include an amplifiable marker on the Reilly vector.

Finally, Applicants point out that there would have been no motivation to place an amplifiable marker on a vector for *in vitro* transcription because amplifiable markers do not function *in vitro*.

For all these reasons, Applicants submit that even if the cited art were to be relied upon as providing the motivation to include an amplifiable marker on the Reilly construct, such reliance would be in error.

In summary, none of the references, alone or in combination, would have suggested introducing the Reilly vector into a cell, integrating the Reilly vector into the genome, or replacing the T7 promoter with a viral or inducible promoter. Reconsideration and withdrawal of the rejection on this basis is, therefore, respectfully requested.

M. The Rejection Under 35 U.S.C. § 103(a)

On page 18 of the Office Action, claims 159 and 167 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Reilly as applied to claims 58, 64, 69, 74, 76, 234–241, 243 and 247 above, and further in view Devine *et al.* (U.S. Patent No. 5,677,170), herein "the '170 patent" and Joshi. Applicants respectfully traverse the rejection.

Claims 159 and 167 depend from claims 58 and 59. Applicants respectfully submit that the references, alone or in combination, do not render these claims obvious. Applicants have discussed the deficiency of Reilly as a primary reference. Reilly does not

disclose or suggest an amplifiable marker. The '170 patent and Joshi do not disclose or suggest an amplifiable marker. Accordingly, for this reason alone, the references do not suggest the claimed invention.

This argument notwithstanding, Applicants respectfully submit that it would not been obvious to include transposition signals in the Reilly vector because, as discussed above, there would have been no motivation to integrate the vector, contrary to the Examiner's assertion.

The Examiner indicates that the motivation to add transposition signals comes from Joshi and the '170 patent. On page 19 of the Office Action, the Examiner states that "it would have been obvious. . .to modify the vector of Reilly et al. by including transposition sequences of Devine. . .and use such vectors in *cloning new genes* by the method of Joshi or *identify splice sites in DNA fragments or in genomic sequences in a cell.*" The '170 patent is relied upon for disclosing methods for creating artificial transposons and inserting these transposons into plasmid targets *in vitro*. Joshi is relied upon for teaching transposon mutagenesis for cloning new bacterial genes. The Examiner cites the last paragraph in column 2 on page 242.

The Examiner reasons that it would have been obvious to provide transposition signals on the Reilly vector and use it to perform insertional mutagenesis in order to identify new genes. Applicants respectfully disagree. The Reilly vector is a specialized vector designed

specifically to assess splicing. The mere fact that the art teaches transposable elements to clone genes fails to provide the suggestion to put the specialized Reilly vector to a completely different use. Without a suggestion that the vector be modified and used for transposition, Applicants respectfully submit that the rationale for this conclusion is in error.

With respect to the motivation to "identify splice sites in DNA fragment. . .," Applicants point out that the vector is already designed for and fully adequate for identifying a splice site in a DNA fragment. The identification of a splice site in a DNA fragment cloned into the multiple cloning site would not be further enhanced by the addition of transposition signals. Accordingly, there would have been no motivation to add such signals for the purpose of identifying splice sites in a DNA fragment.

Finally, a further "motivation" provided by the Examiner is that transposition sequences would allow the identification of splice sites in genomic sequences in a cell. Applicants disagree. The vector is specifically designed to identify splice sites in fragments cloned *into* the vector. There is no suggestion in any of the references to use the Reilly vector to identify splice sites in the genome. If the Examiner disagrees, Applicants request that he point out where the suggestion occurs. Accordingly, this "motivation" to integrate the vector is lacking.

Claim 167 is directed to the vector in a host cell. The Reilly vector, if introduced into a cell, as the Examiner suggested above (in the rejection numbered 26), would have

contained not only the splice donor on the vector, but also a genomic fragment with a splice acceptor. Accordingly, the vector would contain a paired splice donor/splice acceptor. This being the case, the vector would not, in fact, be useful for identifying splice sites outside of the vector in flanking DNA.

Accordingly, contrary to the Examiner's assertion, it would not have been obvious to introduce the Reilly vector into a cell to identify splice sites in other than the cloned genomic fragment on the vector.

In view of the above discussion, Applicants respectfully submit that the references, alone or in combination, would not have suggested Applicants' claimed invention. Moreover, Applicants submit that a combination of the references would not even produce Applicants' invention because not all of the required elements are found (e.g., amplifiable marker). Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

N. The Rejection Under 35 U.S.C. § 103(a)

On page 19 of the Office Action, claims 159 and 167 have also been rejected under 35 U.S.C. § 103(a) on the grounds that they are unpatentable over Treco as applied to claims 59, 69, 71, 73, 74, 76–78, 80–82, 87–89, 109, 114, 116–123, 129, 157, 159 [*sic*], 161, 162, 164–166, 167 [*sic*], 169–175, 177–183, 223, 224, 232 and 233, and further in view of Devine and Joshi. Applicants respectfully traverse the rejection.

As indicated with respect to the rejection under Section 102(e) over Treco, the claims as currently written distinguish over Treco by failing to contain targeting sequences. Accordingly, nothing in Treco would suggest building a vector comprising a transcriptional regulatory sequence, amplifiable marker and viral origin of replication, wherein the vector lacks targeting sequences. Accordingly, the primary reference is deficient. For this reason alone, the teachings of Treco, as previously used in the Office Action, cannot be relied upon.

Moreover, the Examiner takes the position that it would have been obvious to modify the *targeted* vector of Treco and use the vector for random integration to clone new genes by the method of Joshi or identify splice sites in DNA fragments or in genomic sequences. First, Applicants point out that there is nothing in Treco, Devine, or Joshi to suggest using the Treco vectors identify splice sites in DNA fragments or in genomic sequences. If the Examiner believes that such a teaching exists in any of these references, Applicants request that he point out where the references contain this teaching. Applicants request that the Examiner explain how the Treco vectors would even be used to identify splice sites in DNA fragments or in genomic sequences. This motivation appears to be in error, as the paragraph appears to be copied from page 19 of the Office Action, substituting Treco for Reilly. Accordingly, Applicants believe that the Examiner only asserts in this case that it would been obvious to convert the vector of Treco to one including transposition sequences that inserts by random integration. Applications respectfully disagree with this conclusion.

The vectors of Treco were designed in order to modify expression in targeted genes and, not to clone new genes. What the Examiner suggests is not merely a modification of the vector, but a complete change in intended function. Moreover, the vectors of Treco were specifically designed as targeting vectors and to include transposition sequences would defeat the purpose of these vectors. Applicants respectfully submit that there is nothing in the references, alone or in combination, that would suggest modifying the Treco vector to perform a completely different function, and to modify the vector such that the original function is abolished. Accordingly, Applicants respectfully submit that the rejection is improper. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

CONCLUSION

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants, therefore, respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided. Prompt and favorable consideration of this Response is respectfully requested.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 CFR §1.136(a), and any fees required therefore are hereby authorized to be charged to Deposit Account No. **50-0622**, referencing Attorney Docket No. **0221-0003**.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

58. (Once amended) A vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence and one or more sequences encoding an amplifiable ~~markers~~ marker, wherein said vector construct does not comprise a homologous targeting sequence.

59. (Once amended) A vector construct comprising a transcriptional regulatory sequence, a sequence encoding an amplifiable marker, and a viral origin of replication, wherein said vector construct does not comprise a homologous targeting sequence.

73. (Once amended) The cell of claim 71 ~~or 72~~, wherein an endogenous gene is over-expressed in said cell by upregulation of the gene by said transcriptional regulatory sequence on said vector construct.

77. (Twice amended) A method for producing an expression product of an endogenous ~~cellular~~ gene or portion thereof comprising:

(a) introducing the vector construct of either claim 58 or 59 into a eukaryotic cell;

(b) integrating said vector construct into the genome of said cell by non-homologous recombination; and

(c) over-expressing said endogenous gene in said cell.

81. (Twice amended) A cell library comprising a collection of eukaryotic cells transformed with the vector construct of claim 58 or 59, wherein said vector construct is integrated into the genomes of said cells by non-homologous recombination.

85. (Once amended) A method for over-expressing an endogenous gene in a cell *in vivo*, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into a eukaryotic cell *in vitro*;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) introducing said isolated and cloned cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

86. (Once amended) A method for producing an expression product of an endogenous ~~cellular~~ gene *in vivo*, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence into a eukaryotic cell *in vitro*;

(b) integrating said vector integrate into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) introducing said isolated and cloned cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

87. (Once amended) A method for producing an expression product of an endogenous ~~cellular~~ gene, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence and one or more amplifiable markers into a eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene;

(e) culturing said cell under conditions in which said vector and said endogenous gene are amplified in said cell; and

(f) culturing said cell under conditions favoring the production of the expression product of said endogenous gene by said cell.

89. (Twice amended) The method of claim 87 ~~or 232~~, wherein said vector further comprises a splice donor site operably linked to said transcriptional regulatory sequence.

98. (Once amended) A method for over-expressing an endogenous gene in a cell *in vivo*, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence and one or more amplifiable markers into a eukaryotic cell *in vitro*;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) introducing said isolated and cloned cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

109. (Once amended) A method for producing an expression product of an endogenous gene in a cell comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) culturing said cell in reduced serum medium.

110. (Once amended) A method of protein discovery comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) culturing said cell in reduced serum medium under conditions that allow over-expression of an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence, thereby producing cell-conditioned media; and

(d) screening said cell-conditioned media for the presence of the expression product of said gene or portion thereof.

113. (Once amended) A method for producing an expression product of an endogenous ~~cellular~~ gene comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into a eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing ~~an~~ said endogenous cellular gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said ~~endogenous~~ gene; ~~and~~

(e) culturing said cell under conditions favoring the production of the expression product of said ~~endogenous~~ gene by said cell; and

(f) isolating said expression product from a cell mass equivalent to at least 10 liters of cells at 10^4 cells/ml.

116. (Once amended) A method for increasing expression of an endogenous gene in a cell *in situ*, the phenotype of said endogenous gene being known, without making use of any sequence information of the gene, the method comprising the steps of:

(a) constructing a vector comprising an amplifiable marker, a transcriptional regulatory sequence, and an unpaired splice donor sequence;

(b) delivering copies of the vector to a plurality of eukaryotic cells;

(c) culturing the cells under conditions permitting nonhomologous recombination events between the inserted vector and the genome of the cells;

(d) screening the non-homologously recombinant cells by assay for the phenotype of said endogenous gene to identify cells in which the expression of said gene has been enhanced; and

(e) selecting for cells with increased expression of said amplifiable marker and said endogenous gene.

118. (Twice amended) An isolated eukaryotic cell comprising in its genome an inserted genetic construct, said genetic construct comprising an amplifiable marker and a transcriptional regulatory sequence, wherein said genetic construct is inserted into a gene or an upstream region of a gene and

activates the expression of said gene, and wherein said gene and upstream region of said gene have no nucleotide sequence homology required for said insertion to said genetic construct.

157. (Once amended) A vector construct comprising:

(a) a first promoter operably linked to a sequence encoding a positive selectable marker;

(b) a second promoter operably linked to a sequence encoding a negative selectable marker; and

(c) an unpaired splice donor site,

wherein said positive and negative selectable markers and said splice donor site are oriented in said vector construct in an orientation that, when said vector construct is integrated into the genome of a eukaryotic host cell in such a way that an endogenous gene in said genome is transcriptionally activated, then said positive selectable marker is expressed in active form and said negative selectable marker is either not expressed or is expressed in inactive form.

159. (Twice amended) The vector construct of any one of claims 58, 59, and 157, said vector further comprising one or more transposition signals.

161. (Twice amended) The vector construct of any one of claims 58, 59, and 157, said vector further comprising one or more viral origins of replication.

162. (Twice amended) The vector construct of any one of claims 58, 59, and 157, said vector further comprising one or more viral replication factor genes.

165. (Twice amended) The vector construct of any one of claims 58, 59, and 157, said vector further comprising genomic DNA.

166. (Twice amended) A eukaryotic host cell comprising the vector construct of any one of claims 58, 59, and 157.

167. (Once amended) A eukaryotic host cell comprising the vector construct of claim 159.

169. (Once amended) A eukaryotic host cell comprising the vector construct of claim 161.

170. (Once amended) A eukaryotic host cell comprising the vector construct of claim 162.

171. (Once amended) A eukaryotic host cell comprising the vector construct of claim 165.

174. (Twice amended) A library of eukaryotic cells comprising the vector construct of any one of claims 58, 59, and 157.

175. (Once amended) A library of eukaryotic cells comprising the vector construct of claim 159.

177. (Once amended) A library of eukaryotic cells comprising the vector construct of claim 161.

178. (Once amended) A library of eukaryotic cells comprising the vector construct of claim 162.

179. (Once amended) A library of eukaryotic cells comprising the vector construct of claim 165.

180. (Twice amended) A method for activation of an endogenous gene in a cell comprising:

(a) transfecting a genome-containing eukaryotic cell with the vector of any one of claims 58, 59, and 157; and

(b) culturing said cell under conditions suitable for non-homologous integration of said vector into the genome of said cell, wherein said integration results in the activation of ~~an~~ said endogenous gene in the genome of said cell.

181. (Twice amended) A method for identifying a gene comprising:

(a) transfecting a plurality of genome-containing eukaryotic cells with the vector of any one of claims 58, 59, and 157;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of ~~the host~~ said ~~cell~~ cells;

(c) selecting for cells in which said vector has integrated into the genomes of said cells to produce selected cells;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA; and

(f) identifying a gene in said cDNA by isolating one or more cDNA molecules containing one or more nucleotide sequences from said vector.

232. (Once amended) A method for producing an expression product of an endogenous ~~cellular~~ gene, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence and one or more amplifiable markers into a eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing ~~an~~ said endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene;

(e) culturing said cell under conditions in which said vector and said endogenous gene are amplified in said cell; and

(f) introducing said ~~isolated and cloned~~ cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

234. (Once amended) A vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence wherein said vector construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said vector construct does not contain a targeting sequence, and wherein there is no selectable marker between the transcriptional regulatory sequence and the splice donor sequence, wherein said transcriptional regulatory sequence is selected from the group consisting of a cellular transcriptional regulatory sequence and a eukaryotic viral transcriptional regulatory sequence.

235. (Once amended) A vector construct comprising a promoter operably linked to an unpaired splice donor sequence wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein there is no selectable marker between the transcriptional regulatory sequence and the splice donor sequence, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

236. (Once amended) A vector construct comprising a transcriptional regulatory sequence operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor

sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said vector construct does not contain a targeting sequence, and wherein said exon does not contain a selectable marker coding sequence, wherein said transcriptional regulatory sequence is selected from the group consisting of a cellular transcriptional regulatory sequence and a eukaryotic viral transcriptional regulatory sequence.

237. (Once amended) A vector construct comprising a promoter operably linked to an exon, said exon defined at the 3' and by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein said exon does not contain a selectable marker, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

238. (Once amended) A vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence wherein said vector construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said vector construct does not contain a targeting sequence, and wherein there is no internal ribosome entry site between the transcriptional regulatory sequence and the splice donor sequence, wherein said transcriptional regulatory sequence is selected from the group consisting of a cellular transcriptional regulatory sequence and a eukaryotic viral transcriptional regulatory sequence.

239. (Once amended) A vector construct comprising a promoter operably linked to an unpaired splice donor sequence wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein there is no internal ribosome entry site between the transcriptional regulatory sequence and the splice donor sequence, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

240. (Once amended) A vector construct comprising a transcriptional regulatory sequence operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said vector construct does not contain a targeting sequence, and wherein said exon does not contain an internal ribosome entry site, wherein said transcriptional regulatory sequence is selected from the group consisting of a cellular transcriptional regulatory sequence and a eukaryotic viral transcriptional regulatory sequence.

241. (Once amended) A vector construct comprising a promoter operably linked to an exon, said exon defined at the 3' and by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein said exon does not contain an internal ribosome entry site, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

243. (Once amended) A vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, and wherein said splice donor sequence is derived from a naturally-occurring eukaryotic splice donor sequence, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

244. (Once amended) A vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, and wherein said splice donor sequence is derived from a naturally-occurring eukaryotic splice donor sequence, said vector construct further comprising a marker sequence operably linked to a promoter other than the promoter operably linked to said exon.

245. (Once amended) A retrovirus vector construct comprising a first and second retrovirus long terminal repeat sequence, a eukaryotic promoter operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, or is not a reporter gene,

wherein said splice donor sequence is derived from a naturally-occurring eukaryotic gene, and wherein the vector construct does not contain a poly-adenylation site operably linked to said promoter.

246. (Once amended) A retrovirus vector construct comprising a first and second retrovirus long terminal repeat sequence, a eukaryotic promoter operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein said splice donor sequence is derived from a naturally-occurring eukaryotic gene, and wherein the vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein the promoter, exon, and splice donor sequence are present in the vector construct between the long terminal repeat sequences in opposite orientation to the long terminal repeat sequences.

247. (Once amended) A vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

248. (Once amended) A vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not

contain a targeting sequence, and wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, said vector construct further comprising a marker sequence operably linked to a promoter other than the promoter operably linked to said exon.

249. (Once amended) A retrovirus vector construct comprising a first and second retrovirus long terminal repeat sequence, a eukaryotic promoter operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, or is not a reporter gene, and wherein the vector construct does not contain a poly-adenylation site operably linked to said promoter.

250. (Once amended) A retrovirus vector construct comprising a first and second retrovirus long terminal repeat sequence, a eukaryotic promoter operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein the vector construct does not contain a poly-adenylation site operably linked to said promoter, and wherein the promoter, exon, and splice donor sequence are present in the vector construct between the long terminal repeat sequences in opposite orientation to the long terminal repeat sequences.

253. (Once amended) A method of generating a library of eukaryotic cells comprising introducing a vector construct according to any one of claims 234–241 and 243–250 into eukaryotic cells to produce said library.

254. (Once amended) A method of generating a library of eukaryotic cells comprising introducing a vector construct according to claim 242 into eukaryotic cells to produce said library.

257. (Once amended) A method to activate expression of a gene in an isolated eukaryotic cell comprising introducing a vector construct into said cell, wherein said construct comprises a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a targeting sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein said splice donor sequence is derived from a naturally-occurring eukaryotic splice donor sequence, wherein the vector construct is incorporated into the genome of said eukaryotic cell by non-homologous recombination and wherein said splice donor sequence is spliced to a splice acceptor sequence in said activated gene in said isolated eukaryotic cell.

258. (Once amended) A method to activate expression of a gene in an isolated eukaryotic cell comprising introducing a vector construct into said cell, wherein said construct comprises a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a targeting sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein the vector construct is incorporated into the genome of said eukaryotic cell by non-homologous recombination and wherein said splice donor sequence is spliced to a splice acceptor sequence in said activated gene in said isolated eukaryotic cell.

259. (Once amended) The method of claim ~~254~~ 257 wherein said vector construct is a retrovirus vector construct.